Topical Review

Physiological roles for amyloid β peptides

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Alzheimer's disease is recognized *post mortem* by the presence of extracellular senile plaques, made primarily of aggregation of amyloid β peptide (Aβ). This peptide has consequently been regarded as the principal toxic factor in the neurodegeneration of Alzheimer's disease. As such, intense research effort has been directed at determining its source, activity and fate, primarily with a view to preventing its formation or its biological activity, or promoting its degradation. Clearly, much progress has been made concerning its formation by proteolytic processing of the amyloid precursor protein, and its degradation by enzymes such as neprilysin and insulin degrading enzyme. The activities of Aβ, however, are numerous and yet to be fully elucidated. What is currently emerging from such studies is a diffuse but steadily growing body of data that suggests Aβ has important physiological functions and, further, that it should only be regarded as toxic when its production and degradation are imbalanced. Here, we review these data and suggest that physiological levels of Aβ have important physiological roles, and may even be crucial for neuronal cell survival. Thus, the view of Aβ being a purely toxic peptide requires re-evaluation.

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Aβ formation

It is now over two decades since amyloid β peptide (Aβ) was first sequenced and recognized as a potential marker of Alzheimer's disease (Glenner & Wong, 1984). Soon after, this 39–43 amino acid peptide was identified as the major component of the extracellular plaques that define this major form of dementia (Wong *et al.* 1985). Since then a wealth of academic and commercial research has been aimed at understanding where this peptide comes from, for a very simple reason; if we can stop its production, we might also prevent Alzheimer's disease. Alzheimer's disease is a major problem, and one likely to grow with an ageing population since it is primarily a disease of old age. The intensity and volume of dedicated research has resulted in a detailed current understanding of Aβ production which is summarized in Fig. 1. In essence, Aβ is a cleavage product of a large, transmembrane protein, termed APP (amyloid precursor protein). APP can undergo cleavage down one of at least two pathways. In the first pathway, cleavage by the enzyme α-secretase prevents Aβ formation, and instead produces the neuroprotective sAPPα fragment. However, if sequential cleavage by β- and then γ-secretases predominates, Aβ is formed. This peptide can then aggregate over time to produce senile plaques, during which period it also evokes numerous neurotoxic effects (some of which may require a degree of oligomerization), or it can be degraded by enzymes such as neprilysin, insulin degrading enzyme or endothelin converting enzyme (Turner *et al.* 2004). Clearly, the net balance of production *versus* degradation and clearance will determine levels present in cerebrospinal fluid (CSF), which are low but nevertheless measurable even in individuals showing no signs of dementia whatsoever (Selkoe & Schenk, 2003). The presence of Aβ in the CSF of non-demented individuals and in media from neuronal cell cultures (Tamaoka *et al.* 1997; Haass *et al.* 1992) indicates that, as well as having a potential pathological role in Alzheimer's disease, Aβ has a role in the normal physiology of the central nervous system.

The physiology of APP processing

The production and degradation of Aβ has given us many insights into potential target processes for therapeutic intervention aimed at preventing Aβ formation or accelerating its degradation. Certainly, numerous compounds have been designed to interfere with either the β- or γ-secretases, but progress has been hampered by the fact that these enzymes also cleave other substrate proteins, so it is not only the production of Aβ that would be impaired (see reviews by Selkoe &

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Schenk, 2003; Vardy *et al.* 2005). Furthermore, although the potential for beneficial intervention remains great, it must be remembered that this proteolytic pathway is a *physiological* process – only when net Aβ levels become excessive can this process be regarded as pathological. One major, and often overlooked, aspect of APP processing is that numerous peptides can be generated. These – and what is known of their roles – are summarized in Table 1. Clearly, there is much more to learn about the roles and activities of these peptides, but these are beyond the scope of this review. Instead, we will focus on the potential physiological roles of Aβ. Such a discussion is complicated by the fact that many studies on the effects of Aβ focus on the toxic actions of the peptide. Concentrations of Aβ used in such studies are often far higher than levels found in CSF (1–10 nM). We have therefore directed our attention to studies where levels of applied Aβ are in the < 100 nM range, or where endogenous Aβ production/breakdown has been modified.

Physiological control of synaptic activity

Several lines of evidence indicate that Aβ may have a role in controlling synaptic activity. Kamenetz *et al.* (2003) found that evoked activity of hippocampal neurones in brain slices increased the production of Aβ primarily by increasing trafficking of APP towards β secretase sites at the cell membrane. This would promote Aβ formation, but also increase production of other fragments (Fig. 1) such as AICD which may also modulate synaptic activity. At physiological expression levels of APP, this provided a negative feedback, since Aβ depresses synaptic activity. Without such depression, synaptic activity could become excessive, leading to excitotoxicity. Indeed, γ

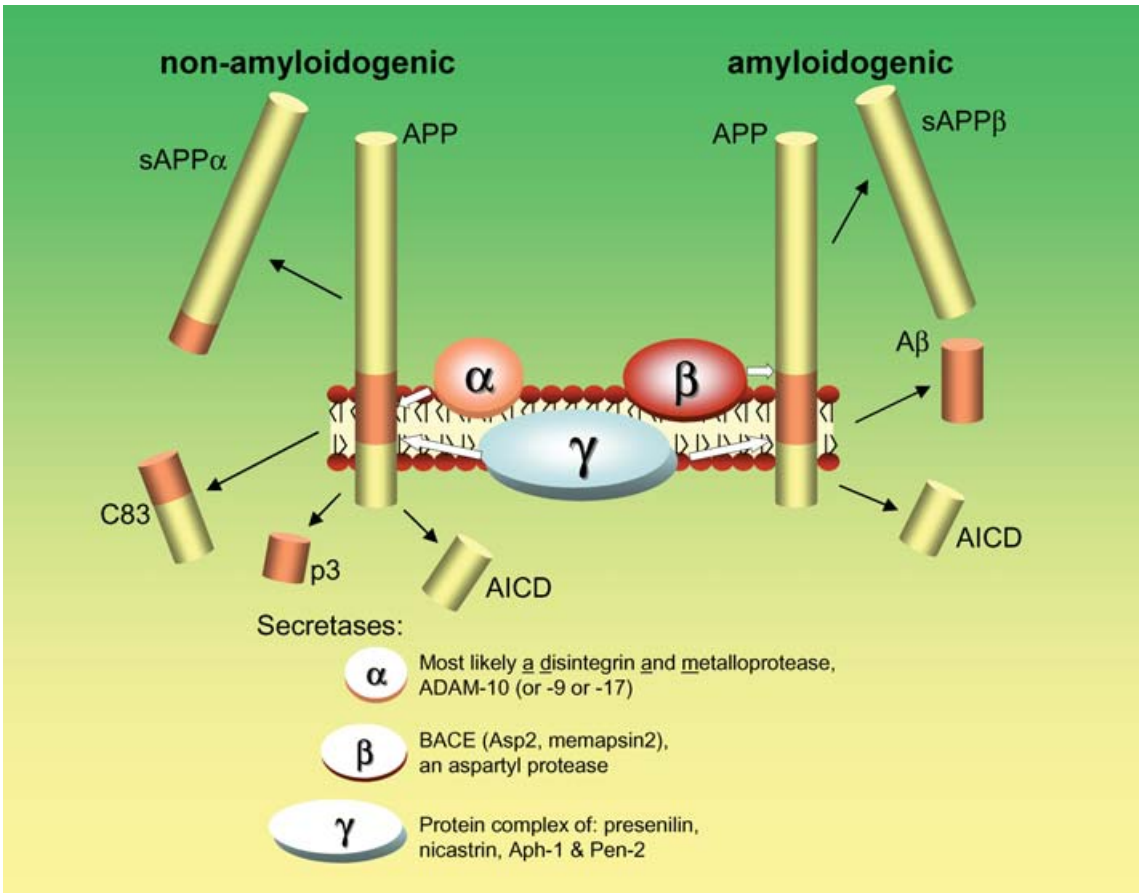

Figure 1. Cartoon depicting the proteolytic processing of amyloid precursor protein (APP) via non-amyloidogenic (left) and amyloidogenic (right) cleavage
Non-amyloidogenic cleavage occurs when α-secretase acts to liberate sAPPα and C83, the latter being cleaved by γ-secretase to generate p3. Amyloidogenic cleavage by β-secretase liberates sAPPβ and the residual peptide is cleaved to produce C88 and Aβ. Aβ in turn can be degraded by enzymes including neprilysin, insulin degrading enzyme and endothelin cleaving enzyme (not shown).

Table 1. Cleavage products of amyloid precursor protein (APP) and their suggested physiological roles

Peptide	Cleavage process	Known function	Reference
sAPPα	α-secretase cleavage of APP	Neuroprotective? Involved in blood clotting? Up-regulates BK_{Ca} activity	Smith *et al.* 1990 Furukawa *et al.* 1996
C83	α-secretase cleavage of APP	None known, but may be associated with neurodegeneration	Rockenstein *et al.* 2005
p3	γ-secretase cleavage of C83	None known?	Pardossi-Piquard *et al.* 2005
AICD/CFTγ	γ-secretase cleavage of C83	Transcriptional regulation (e.g. of neprylisin)	Leissring *et al.* 2002
sAPPβ	β-secretase cleavage of APP	Neuroprotective?	
C99	β-secretase cleavage of APP	Altered acetylcholinesterase activity and behavioural influences	Dumont *et al.* 2005
Aβ	γ-secretase cleavage of β-secretase cleaved of APP	Depress synaptic activity Regulation of K_v expression Regulation of Ca_v	Kamenetz *et al.* 2003 Ramsden *et al.* 2001; Plant *et al.* 2005 Ramsden *et al.* 2002

secretase inhibition led to increased EPSC frequency (Kamenetz *et al.* 2003), and kainate-induced seizures are potentiated in APP knockout mice (Steinbach *et al.* 1998). Further to these studies, a recent report indicated that specific stimulation of NMDA receptors up-regulated APP, inhibited α-secretase activity and promoted Aβ production (Lesne *et al.* 2005). Collectively, these studies argue strongly that APP processing, and the presence of Aβ itself, are closely associated with synaptic activity and may serve to provide physiological control of activity, guarding against excessive glutamate release.

What happens in the absence of Aβ?

In primary cultures of central neurones inhibition of endogenous Aβ production (by exposure to inhibitors either of β- or γ-secretases) or immunodepletion of Aβ caused neuronal cell death (Plant *et al.* 2003). Importantly, this appeared neurone-specific, since a variety of non-neuronal cells were unaffected by the same treatments. Perhaps most important, however, was the fact that neuronal cell death in response to secretase inhibition could be restored by addition of physiological (picomolar) levels of Aβ. The most common isoform, $A\beta_{(1-40)}$, was most effective in this regard, and the commonly used fragment $A\beta_{(25-35)}$, which retains many of the toxic properties of Aβ, was almost completely ineffective (Plant *et al.* 2003). These findings provided compelling evidence for a role for Aβ in neuronal survival. The underlying mechanism remains to be determined, but may involve altered expression of K^+ channels. The activity of various K^+ channel types has been implicated in neuronal survival or death, in part because they govern excitability and hence the excitotoxicity of released glutamate, but also because intracellular $[K^+]$ is a key determinant of apoptosis (Yu, 2003). Changes in K^+ channel expression may also provide an explanation of the findings of Kamenetz *et al.* (2003). We recently reported that inactivating K^+ currents of central neurones are suppressed in amplitude by inhibition of the production of endogenous Aβ (Plant *et al.* 2005). As previously, this effect could be recovered by low levels of exogenous Aβ. This therefore probably reflects a physiological role for Aβ in controlling both excitability and cell survival.

We would predict from these findings that transgenic animals in which APP expression had been knocked out would show severe neurological deficits or lethality. This is not the case. APP-null mice show reduced branching of dendrites and fewer synaptic boutons but no reduction in neuronal number despite an absence of Aβ (Dawson *et al.* 1999). Furthermore, these reported neurological changes may not be a result of a deficiency in Aβ but of a deficiency in another product of APP processing or of APP itself. This is supported by the fact that an APP/APLP (amyloid precursor like protein) double knockout is lethal (von Koch *et al.* 1997). In addition, a transgenic knockout of β-secretase in which levels of Aβ are reduced to less than 10% of controls show no obvious deficits in either behaviour or neurology (Luo *et al.* 2001). On the other hand, one could argue that compensatory mechanisms in APP-null transgenic animals prevent toxic changes from occurring. One approach that would answer this would be to test the effects of an APP conditional knockout, but as far as we are aware these have yet to be made.

Hypoxia and APP processing

It has long been known that individuals who have suffered severe or chronic periods of hypoxia are more likely to develop Alzheimer's disease subsequently (Desmond *et al.* 2002). Indeed, hypoxic/ischaemic conditions up-regulate APP mRNA and protein (Hall *et al.* 1995; Kokmen *et al.* 1996; Jendroska *et al.* 1997; Shi *et al.* 2000), with consequent increases in Aβ. *In vitro*, hypoxia increases Aβ production (Taylor *et al.* 1999) with numerous consequences for cell function. Interestingly, many of these concern Ca^{2+} signalling (Smith *et al.* 2003, 2004),

and disruption of Ca^{2+} signalling is a key event underlying neuronal death in Alzheimer's disease (LaFerla, 2002). One important aspect is the alteration of functional expression of ion channels: in cerebellar granule neurones, prolonged hypoxia leads to a selective up-regulation of L-type Ca^{2+} channels. This effect requires Aβ formation, since it could be prevented by secretase inhibitors (Webster *et al.* 2006). This mechanism was explored further using a recombinant expression system: results (summarized as part of the cartoon of Fig. 2) indicated that hypoxia triggered increased Aβ production. This effect was dependent on an increase of reactive oxygen species (ROS) derived specifically from mitochondria (this in itself is seemingly paradoxical, but continues to gain increasing support; see Guzy *et al.* 2005). Once formed, the Aβ interacted directly with the recombinant L-type Ca^{2+}

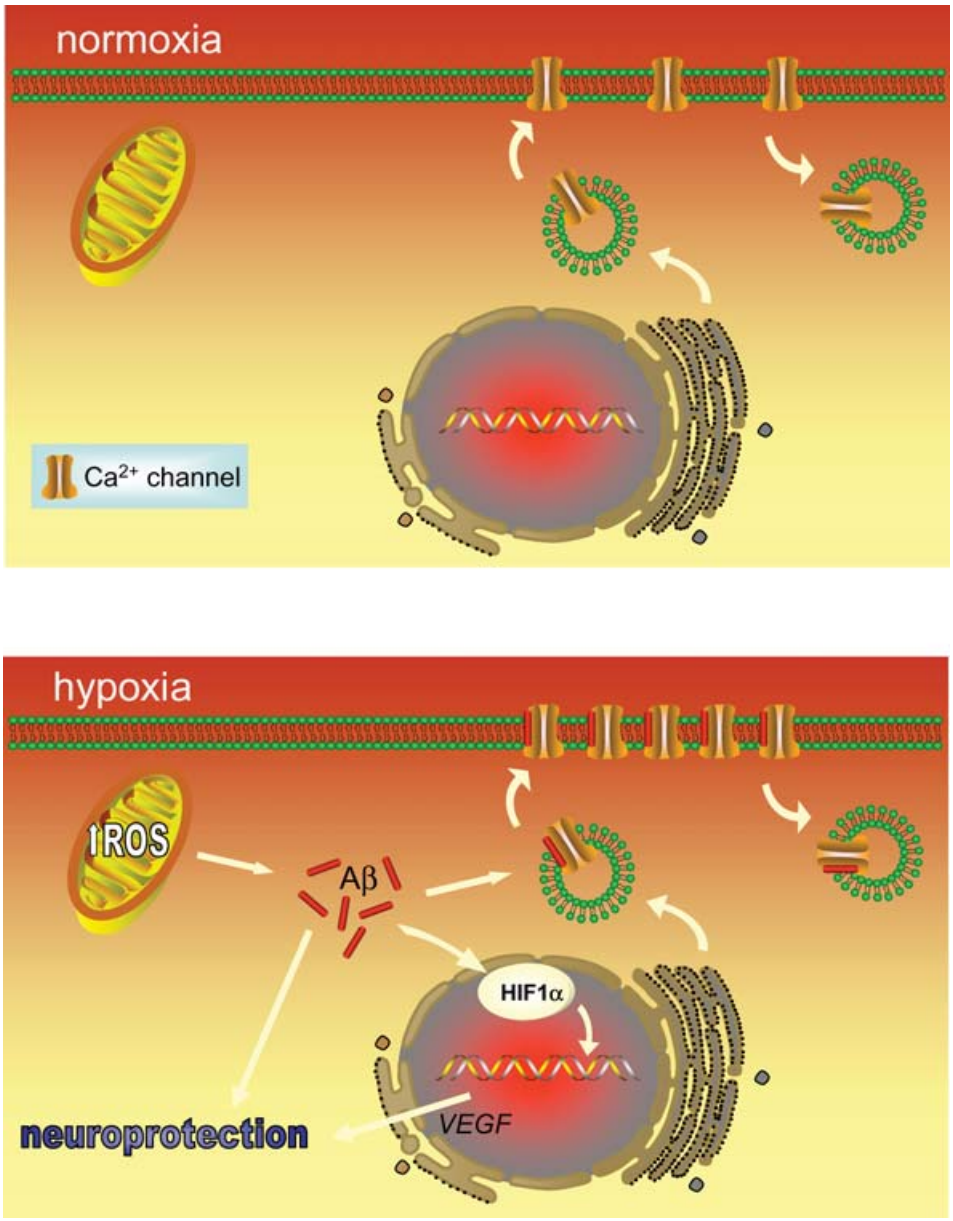

Figure 2. Synthesis, trafficking and retrieval of Ca^{2+} channels from the plasma membrane
Cartoon depicting the synthesis, trafficking and retrieval of Ca^{2+} channels from the plasma membrane under normoxia (upper picture). During sustained hypoxia, a rise of ROS from mitochondria triggers increased Aβ formation which has multiple effects (see text for full description) including direct neuroprotection, increased transcription of protective factors such as vascular endothelial growth factor (VEGF) via stabilization of HIF, and altered trafficking of Ca^{2+} channels so that more are present and active in the plasma membrane.

channel (α_{1C}) subunit either to promote trafficking of channels towards, or inhibit retrieval of channels from, the plasma membrane. The net effect was increased Ca^{2+} channel protein at the cell membrane and hence increased Ca^{2+} conductance.

The question arises, however, of whether this altered channel trafficking represents a physiological or pathological response of cells to hypoxia. On one hand, it is convenient to propose the idea of altered ion channel trafficking as a mechanism accounting for the clinical observations that prolonged hypoxia promotes development of Alzheimer's disease (see earlier). Alternatively, this can be regarded as a physiological process. This work – like the vast majority of *in vitro* cellular studies – was conducted using a 'normoxic' level of 150–160 mmHg (i.e. room air-equilibrated conditions). This is, of course, hyperoxic for any cell. For central neurones, this is a particularly high ambient level of O_2. Central nervous system O_2 levels vary, but rarely exceed approximately 40 mmHg (Li *et al.* 2005). Indeed, commonly, they can be half this value, as measured in animals breathing normal air (Grote *et al.* 1996). A compelling recent study has indicated that if neurones are isolated and maintained in primary culture at *physiological* O_2 levels, they appear more robust, and can tolerate hypoxia to much greater levels than cells cultured at 20% O_2 (Li *et al.* 2005). The protection appeared to derive in part from the production of VEGF (vascular endothelial growth factor), which in turn was driven by the stabilization of the α1 subunit of the transcriptional regulator, hypoxia inducible factor (HIF-1α). Indeed, pharmacological activation of HIF-1α promoted neuronal survival, as did exogenous VEGF (Li *et al.* 2005). But do these effects involve Aβ in any way? At present we simply do not know, but available evidence points to a potentially exciting link. Soucek *et al.* (2003) examined the relationship between HIF-1, Aβ and glucose metabolism in central neurones particularly with a view to examining potentially protective shifts in metabolic activity (generating greater reducing equivalents via glycolysis and the hexose monophosphate shunt). Crucially, they found that low levels of Aβ could induce HIF-1 and thereby protect cells from the toxicity of exposure to high levels of Aβ. In addition, activation of HIF also mimicked the protective effects of low levels of Aβ. The authors themselves concluded that 'an early function of Aβ in ageing is neuroprotection' (Soucek *et al.* 2003).

Summary

This brief review has highlighted some of an accumulating but diffuse collection of work pointing to important physiological roles for Aβ. Given the presence of specific enzymatic pathways for the constitutive generation of Aβ, coupled with the fact that there exist selective uptake, breakdown and clearance pathways for its removal, it seems inconceivable that Aβ does not have a role to play in the normal function of the nervous system. Aβ should not, therefore, be regarded merely as a toxic factor that requires eradication to avoid dementia. Clearly, there is evidence to suggest essential modulation of synaptic activity and neuronal survival, but what the full physiological extent of Aβ activity (or indeed the activity of other products of APP proteolysis) remains to be seen.

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